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Cellular and Molecular Toxicity of Lead in Bone

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To fully understand the significance of bone as a target tissue of lead toxicity, as well as a reservoir of systemic lead, it is necessary to define the effects of lead on the cellular components of bone. Skeletal development and the regulation of skeletal mass are ultimately determined by the four different types of cells: osteoblasts, lining cells, osteoclasts, and osteocytes. These cells, which line and penetrate the mineralized matrix, are responsible for matrix formation, mineralization, and bone resorption, under the control of both systemic and local factors. Systemic components of regulation include parathyroid hormone, 1,25-dihydroxyvitamin D_3, and calcitonin; local regulators include numerous cytokines and growth factors.

Lead intoxication directly and indirectly alters many aspects of bone cell function. First, lead may indirectly alter bone cell function through changes in the circulating levels of those hormones, particularly 1,25-dihydroxyvitamin D_3, which modulate bone cell function. These hormonal changes have been well established in clinical studies, although the functional significance remains to be established. Second, lead may directly alter bone cell function by perturbing the ability of bone cells to respond to hormonal regulation. For example, the 1,25-dihydroxyvitamin D_3-stimulated synthesis of osteocalcin, a calcium-binding protein synthesized by osteoblastic bone cells, is inhibited by low levels of lead. Impaired osteocalcin production may inhibit new bone formation, as well as the functional coupling of osteoblasts and osteoclasts. Third, lead may impair the ability of cells to synthesize or secrete other components of the bone matrix, such as collagen or bone sialoproteins (osteopontin). Finally, lead may directly effect or substitute for calcium in the active sites of the calcium messenger system, resulting in loss of physiological regulation. The effects of lead on the recruitment and differentiation of bone cells remains to be established. Compartmental analysis indicates that the kinetic distribution and behavior of intracellular lead in osteoblasts and osteoclasts is similar to several other cell types. Many of the toxic effects of lead on bone cell function may be produced by perturbation of the calcium and cAMP messenger systems in these cells.

Introduction

Importance of Bone Lead

Although the affinity of lead for the skeleton has been widely recognized for many years, clinical, epidemiological, and experimental evaluations of skeletal lead toxicity are relatively rare and generally less sophisticated in approach than assessments of lead toxicity in other target organs such as the nervous system. Nevertheless, lead intoxication produces a diverse array of pathological changes in human and animal skeletons. As is customary with most outcomes of lead intoxication, the effects are dependent upon lead dose, duration of exposure, dietary calcium and phosphorus, and other experimental variables. Furthermore, the manifestation of lead intoxication in bone is undoubtedly the result of complex interplay between systemic endocrine effects, cellular processes in bone, and chemical processes in the bone matrix. Based upon the numerous and diverse effects of lead on the skeleton, the interaction of lead and calciotropic hormones, and the actions of lead on bone cell function, it is likely that as more sophisticated and appropriate experimental designs and measures of skeletal toxicity are applied, adverse effects of chronic, low-level lead will be demonstrated in the skeleton.

It has long been acknowledged that the skeleton contains most of the body burden of lead in humans. The accumulation of lead in the skeleton begins during fetal bone development and continues to age 60 years. The toxicological significance of bone lead is increasingly recognized, although many aspects of bone lead metabolism and toxicity are not clearly characterized. Lead in bone is of scientific and clinical interest for

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three general reasons. First, skeletal lead is important as a reservoir of lead which may be mobilized by physiological and pathological states including pregnancy, lactation, and osteoporosis (*1,2*). The mobilization of lead from these internal stores may cause adverse effects in other tissues, including the fetus. Second, bone lead is important as the most meaningful measure of cumulative lead exposure and most accurate predictor of deficits in neurobehavioral outcomes produced by lead (*3,4*). And finally, the skeleton is increasingly recognized as an important target organ system for lead toxicity. (*5,6*).

There are several conceptual and technical intricacies to establishing the dose-response relationship of lead exposure to skeletal toxicity and to elucidating the cellular and molecular mechanisms of lead toxicity in the bone. Bone is a dynamic tissue, undergoing remodeling throughout life, and is regulated by a wide range of hormonal and local factors. The skeleton and bone mineral metabolism are also altered by physiological states, such as pregnancy and aging or disease. Skeletal lead toxicity, altered bone mineral metabolism, and bone lead metabolism, must then be identified in the context of a complex regulatory system for bone and bone minerals (Fig. 1). This task is complicated by the small volume of cells in bone compared to the much larger matrix volume that is maintained by these cells. In addition, the cells themselves exhibit a remarkable functional heterogeneity, integration, and coordination in the formation and resorption of bone. Furthermore, bone cells occupy a unique microenvironment with respect to lead, in that they are in direct contact with and interact with bone.

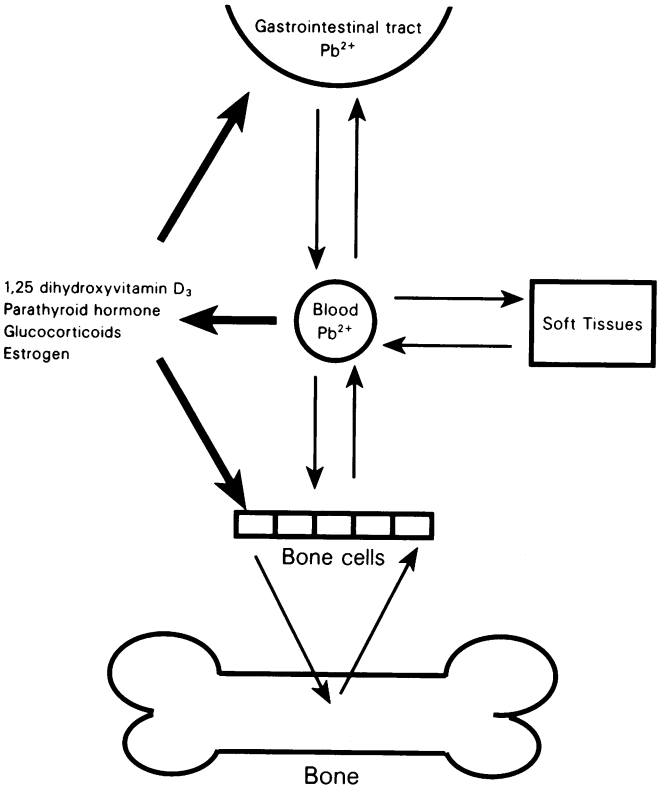

FIGURE 1. Schematic figure illustrating the relationship of important dietary and hormonal factors controlling and affecting normal bone physiology, bone mineral metabolism, and systemic and skeletal lead metabolism.

Overview of Bone Cell Biology

An overview of the relationships of bone cells, osteoid, mineralized matrix, and systemic and local regulators of bone cell function is illustrated in Figure 2. The regulation and function of these bone constituents is described in some detail in the following sections. Skeletal development and the regulation of skeletal mass are ultimately determined by the four different types of cells: osteoblasts, lining cells, osteoclasts, and osteocytes. These cells, which line and penetrate the mineralized matrix, are responsible for matrix formation, mineralization, and bone resorption, under the control of both systemic and local factors. Systemic components of regulation include parathyroid hormone, 1,25-dihydroxyvitamin D_3, and calcitonin; local regulators include numerous cytokines and growth factors. A critical concept in the regulation of bone remodeling is the functional coupling of bone formation and resorption. Most hormonal stimulators of osteoclastic bone resorption including parathyroid hormone do not act directly on osteoclasts, but rather first on osteoblasts, then release soluble factors that mediate osteoclast bone resorption. In turn, osteoclasts generate factors that influence osteoblastic proliferation, migration, differentiation, matrix synthesis, and cessation of synthesis. The documented effects of lead on each of these aspects of bone cell biology will be discussed in turn, including parallels with other organ systems when appropriate. Important gaps in our knowledge and understanding of these processes will be identified as well.

The objective of this review is to provide a perspective concerning the cellular and molecular aspects of bone lead metabolism and toxicity rather than an exhaustive review. Emphasis will be placed on relating the interactions of lead and bone cells in the context of current understanding of bone cell biology and the bone matrix. In addition, we will review the diverse and complex adverse effects of lead in this target organ. Relatively little attention will be paid to the distribution and metabolism of lead in bone.

Pathology of Lead in the Skeleton

Lead intoxication produces an array of effects on both human and animal skeletal systems. These effects include perturbation of bone development, bone formation, and bone resorption. This section will briefly illustrate some of the diverse effects of lead on the skeleton and the interdependency of lead toxicity, calciotropic hormones, and diet. The reader is directed to more comprehensive reviews of bone lead (*7*).

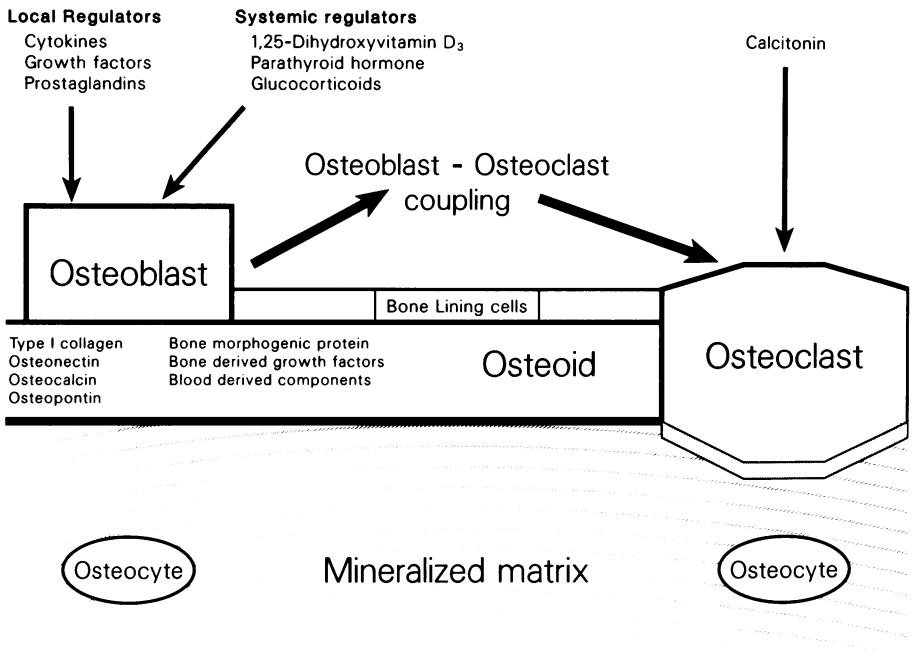

FIGURE 2. Schematic figure illustrating the relationship of the osteoblasts, osteoclasts, lining cells, and osteocytes to the bone matrix and some of the systemic and local factors that regulate bone cell function.

In common with other target organ systems, such as the nervous system, the developing skeleton seems more sensitive to lead than the adult skeleton. Lead readily crosses the placenta and is associated with skeletal malformations in the mouse, rat, and hamster (*8–11*). Most of the abnormalities consist of fusion of two or more vertebrae in the anterior part of the axial skeleton (*12*). With dietary calcium deficiency, these effects are exacerbated, and there is a delayed ossification in lead-exposed fetuses.

Human congenital lead poisoning is associated with overt skeletal toxicity evidenced by dense cranial vault and delayed skeletal and deciduous dental development at birth (*13*). When these children were examined 7 months after chelation therapy, radiographs revealed normal maturation, although tooth eruption did not occur until 15 months of age.

Postnatal growth of children also appears sensitive to chronic, low-level lead intoxication. A detailed analysis of the NHANES II data base by Schwartz et al. (*5*) found an inverse relationship between blood lead levels and height and chest circumference in children less than 7 years of age. These findings have been confirmed in general by other retrospective studies (*14*) and by the preliminary findings of 260 infants in a prospective study (*6*). These studies were not designed to distinguish between endocrine aspects (growth hormones), impaired energy production and utilization (thyroid hormones), and more direct effects on bone cell regulation and function as critical factors in reduced stature. However, recent studies have found reduced plasma levels of osteocalcin, a bone matrix protein which is synthesized by osteoblasts, in lead toxic children (*15*). Serum osteocalcin is a marker of osteoblast activity, although it is not clear if reduced osteocalcin levels are a cause or an effect of impaired bone formation. Nevertheless, these important studies demonstrate impaired skeletal growth postnatally, with blood lead below 10 μg/dL. The underlying mechanism and clinical significance remain to be established.

Perhaps the most complete experimental analysis of the lead intoxicated skeleton is the assessment of bone turnover, formation, and resorption dynamics by morphometric analysis. Anderson et al. (*16*) showed that in beagle dogs exposed to lead for nearly 7 months, lead intoxication decreased appositional rates, radial closure rates, activation frequency, bone formation rate, and increased osteon formation time. These quantitative measures may be interpreted as manifestations of bone formation dynamics at the cellular, tissue, and organ levels. These findings strongly support lead-dependent decreases in bone formation rates at all three levels of skeletal organization. A similar and subsequent study in the same laboratory not only confirmed the previous findings even with a lower lead dose, but also examined the reversibility of these pathological changes (*17*). When beagle dogs were exposed to lead for 6 months followed by cessation of lead exposure for 6 additional months, there was a persistent depression of cellular activity, but not bone formation at the tissue or organ level, as inferred from the histomorphometric measurements. Although these studies were performed on a small number of animals, they provide important and convincing evidence that chronic lead exposure

alters normal bone physiology in adult animals. Other studies, also using beagles, but using less sophisticated radiographic morphometric and bone radiographic densitometric techniques, did not find differences between control and low-level oral lead exposure (*18*).

Impaired bone formation is often associated with impaired formation of one or more constituents of the organic matrix. Early studies by Hass et al. in lead intoxicated rabbits found retarded formation of new bone at epiphyseal plates, which was accompanied by evidence of increased osteoclastic activity (*19*). Microscopically, these changes were evidenced by the delicacy of trabecular structures and excess lacunar resorption of trabeculae adjacent to zones of ossification. These investigators regarded the lesion as primarily one of inhibited osteoid formation complicated by enhanced resorption of mineralized bone. This hypothesis was further explored in rabbits with hypervitaminosis D and confirmed that lead intoxication impaired the production of not just bone matrix but arterial matrix as well (*20*). While estimating human hazard from these studies with superimposed hypervitaminosis is difficult at best, these studies clearly indicate the ability of lead to alter bone growth and production of bone.

Numerous reports have documented focal lead toxicity in regions of retained bullet fragments. Although this rather uncommon route of lead toxicity is of limited use in defining the skeletal toxicity of lead in larger populations exposed to lead via more conventional routes, these studies do confirm that lead intoxication causes death of osteoclasts, induction of nuclear and cytoplasmic lead-containing inclusions, lead-containing mitochondrial precipitates, and defects in bone formation and resorption which may be described as incomplete osteocytic osteolysis (*21*). These selected reports illustrate the diverse effects of lead on the skeleton. The skeletal toxicity of lead parallels that in other organ systems in that the developing system may be more sensitive. Furthermore, skeletal toxicity is modified by factors such as age and diet.

Lead and Systemic Regulation of Bone Cell Function

Bone cells are under systemic regulation by a broad spectrum of hormones (Fig. 2). This hormonal regulation and perturbed regulation is important in normal and pathological disease processes, including osteoporosis. Interaction of lead and systemic regulators of bone function is exceedingly complex. First, plasma levels of several of these hormones are altered in lead intoxication, as discussed below, demonstrating the potential for perturbed systemic regulation of bone mineral metabolism and skeletal function. Second, many of these systemic regulators, such as 1,25-dihydroxyvitamin D_3, also modulate systemic absorption and skeletal metabolism of lead. Finally, lead intoxication may alter cellular response of target bone cells to these systemic regulators. For more complete review of lead and calciotropic hormones, including interactions of lead with the hormonal regulation of calcium absorption and plasma calcium, the reader is directed to earlier reviews (*22*).

Vitamin D

The hormonal form of vitamin D, 1,25-dihydroxyvitamin D_3, is necessary for bone growth and mineralization, primarily for its regulation of serum calcium and phosphorus. Osteoblasts, but not osteoclasts, have 1,25-dihydroxyvitamin D_3 receptors, indicating that stimulation of osteoclastic bone resorption by 1,25-dihydroxyvitamin D_3 probably involves local regulatory mechanisms (discussed below). 1,25-dihydroxyvitamin D_3 exerts its effects at the level of mRNA transcription, which is characteristic of steroid hormones. However, several aspects of 1,25-dihydroxyvitamin D_3 effects on osteoblasts remain unresolved.

Several studies report a significant effect of lead on vitamin D levels in children. A strong negative correlation was observed between the circulating level of 1,25-dihydroxyvitamin D_3 and blood lead levels in children (*23,24*). Plasma 1,25-dihydroxyvitamin D_3 levels in lead intoxicated children were reduced to levels comparable to those of patients with metabolic bone disease, uremia, and hypothyroidism. However, after chelation therapy, plasma 1,25-dihydroxyvitamin D_3 levels returned to normal. Clinical observations are supported by experimental studies that demonstrate depressions of plasma 1,25-dihydroxyvitamin D_3 in rats fed 0.82% lead as lead acetate (*25*). In the same study, lead exposure blocked the intestinal calcium transport in response to administration of vitamin D. These observations are also significant because of the recently recognized role of 1,25-dihydroxyvitamin D_3 in cell differentiation, cell maturation, and immunoregulation.

Parathyroid Hormone

Parathyroid hormone is a potent stimulator of osteoclastic bone resorption through several mechanisms. This hormone stimulates the activity of all enzymes involved in bone resorption in osteoclasts, but not through direct effects on osteoclasts. Osteoclasts do not have parathyroid hormone receptors. Rather, parathyroid hormone interacts with membrane receptors on osteoblasts, which then activate the osteoclastic resorption. Co-culture experiments strongly support the role of an osteoblast-derived soluble paracrine factor that regulates osteoclast activity (*26,27*). These observations illustrate and emphasize the functional coupling of osteoblasts, which have parathyroid hormone receptors, and osteoclasts, which do not. Parathyroid hormone may also affect the proliferation, differentiation, and/or fusion of osteoclast progenitor cells (*28*).

The actions of lead on parathyroid hormone are not fully characterized. The single human study is the clinical study of Rosen and co-workers, who found in-

creased serum parathyroid hormone associated with slightly decreased concentration of ionized calcium in whole blood of lead-intoxicated children (*23*). The effect of parathyroid hormone on renal and bone accumulation of lead has also been accessed in adult rats by Mouw and co-workers (*29*). Subcutaneous injection of parathyroid extract increased both bone and renal lead concentrations of animals exposed to lead in the drinking water. The mechanism and significance of increased parathyroid hormone in humans, or indeed the sensitivity of other populations at risk such as lead workers or the aged, and the sites of lead-parathyroid hormone interactions remain to be established.

Calcitonin

Calcitonin, a small peptide also known as hypocalcemic hormone, is a major inhibitor of bone resorption through direct effects on osteoclastic bone cells. Osteoclasts, but not osteoblasts, have calcitonin receptors, and there are no apparent effects of calcitonin on osteoblasts or bone formation. In osteoclasts, calcitonin causes a transient increase in calcium influx (*30*) and reduces carbonic anhydrase II activity along the ruffled border (*31*). This hormone also causes a decrease in osteoclastic spreading, which also may be related to decreased resorption (*32*). Little is known of the long-term effects of lead on either calcitonin-mediated function or the regulation of calcitonin. Following acute lead exposure, however, calcitonin has been shown to inhibit the hypercalcemia produced by IP or IV injections of high levels of lead (*33–37*). However, since hypocalcemia, not hypercalcemia, is observed with chronic lead intoxication, the significance of these observations to human exposure remains to be established.

Glucocorticoids

Glucocorticoid hormones, both natural and synthetic, are potent simulators of bone resorption *in vivo* and *in vitro*, in addition to producing profound and diverse effects in other organs. The effects of glucocorticoids on bone cell function are apparently mediated through both direct effects on osteoblasts (*38*) and indirectly by enhancing the effects of parathyroid hormone. Despite a few provocative reports, few investigations have explored the potentially important and promising relationships between glucocorticoids and lead toxicity. For example, the urinary excretion of the highly polar metabolite of cortisol, 6β-hydroxycortisol, is reduced in lead-poisoned children, suggesting impaired glucocorticoid metabolism by the cytochrome P-450-dependent enzymes of the liver (*39*). Furthermore, stress, which elevates plasma glucocorticoid levels, has been shown to increase blood lead levels in nonhuman primates, presumably by mobilizing lead stores through stimulated bone resorption (*40*). The potential actions and interactions of lead and glucocorticoids on bone cells, bone resorption, and other effects may be related to or confounded by concurrent induction of genes expressing heat-shock-like proteins, but via different induction mechanisms (*41*).

Lead and Local Regulation of Bone Cell Function

The regulation of bone cell function is the result of a dynamic interplay between systemic and local mediators. The local regulators include paracrine and endocrine factors which may be secreted by local cells, stored in bone matrix, and released from bone matrix during resorption. Local regulation and the coupling of osteoblast and osteoclast function is a new and active area of bone cell biology. Although virtually nothing is known currently concerning the effects of lead on the paracrine and autocrine regulation of bone cells, this should be a very productive area of research in lead toxicology.

Cytokines

Several cytokines (lymphokines) have been shown to have important roles in regulating bone cell function. These local regulators may play a prominent role in both physiological and pathological conditions by mediating the effects of systemic hormones such as parathyroid hormone and 1,25-dihydroxyvitamin D_3 and the coupling of osteoblast and osteoclast function.

Interleukin-1 is a family of polypeptides with widespread immunological and nonimmunological activity. Interleukin-1 β, also known as osteoclast-activating factor, potently stimulates bone resorption and stimulates DNA synthesis and osteoblast proliferation (*28,42*). Interleukin-1 has no effect on bone resorption unless osteoclasts are co-cultured with osteoblasts underscoring the importance of coupled osteoblast-osteoclast function (*43*).

Other cytokines with significant bone activity include tumor necrosis factor-α (TNF-α) and TNF-β (lymphotoxin), which were first described and identified on the basis of their cytotoxic actions on tumor cells. These cytokines have numerous effects on many nonmalignant cells including stimulation of bone resorption by osteoclasts. As is the case with interleukin-1, TNF-α and TNF-β depend on the presence of co-cultured osteoblastic cells, indicating that the cytokines stimulate osteoclastic bone resorption via effects on osteoblasts (*43*).

Nothing is known concerning the effects of lead on cytokine production or the response of bone to local regulation by cytokines. Experiments with macrophages suggest that lead does not interfere with interleukin-1 production (*44*) nor the interaction of interleukin-2 with its receptor (*45*). It is not clear, however, whether lead alters cellular responses to interleukin-1 in bone or any other tissue. Local regulation is made more complex by the synergistic action of TNF-α and interleukin-1 on bone resorption (*46*).

Prostaglandins

Prostaglandins are unsaturated, oxygenated fatty acids that are synthesized from arachidonic acid. Their local production and very short half-time make them ideal local regulators of cell function. The specific effects of prostaglandins on bone function are undoubtedly complex and remains confusing (*28*). Unfortunately, little if anything is known regarding lead and prostaglandins in other tissues. The investigations are very limited, but it appears that lead intoxication has little effect, at least on serum levels of prostaglandins E and F (*12*).

Differentiation and Growth Factors

Because of the short, finite life span of osteoblasts and osteoclasts, it is natural that growth factors play an important role in maintaining the cellular integrity of bone differentiation and local control of bone cell function. Bone morphogenic protein and bone-derived growth factors are the most important and best characterized of the differentiation and growth factors produced by bone (*47*). Bone morphogenic protein irreversibly induces differentiation of mesenchymal cells into osteoprogenitor cells. Bone-derived growth factors are secreted by (and for) the osteoprogenitor cells, which stimulate proliferation of these cells. Nothing is known regarding the effects of lead on these important regulators of bone development, growth, remodeling, and repair. Indeed, little is known regarding the effects of lead on growth factors in any other target organ of lead poisoning.

Direct Effects of Lead on Osteoblasts

The osteoblast has a life time of 10 to 20 days and is derived from a local mesenchymal stem cell, the osteoprogenitor cell, which may differentiate into either a chondroblast or an osteoblast (*28,48*). The osteoblasts elaborate many important constituents of the organic bone matrix or osteoid and initiate the mineralization process. The osteoblast also plays a key role in the regulation of bone resorption. Activation of osteoclastic bone resorption by parathyroid hormone, 1,25-dihydroxyvitamin D_3, or cytokines requires osteoblasts to produce, as yet undefined, local factors that activate osteoclastic bone resorption. This functional coupling of osteoblasts and osteoclasts is one of the more important aspects of bone cell physiology. The reader is directed to more comprehensive reviews of the origin, properties, function, and fate of osteoblasts (*28,49*).

Osteoprogenitor Cell Recruitment

The osteoblast is a relatively short-lived cell. Most studies suggest that this cell type lasts about 10 to 14 days and is replaced by cell division and differentiation of progenitor cells. Accordingly, the osteoblastic bone population is analogous to developmental systems in that cell division and biochemical and morphological differentiation are key processes. Since developing and actively growing animals and humans and developing organ systems such as the nervous system are generally considered to be more susceptible to lead poisoning (*50*), these processes may also be targets of lead poisoning even in adults. No experimental data are available that directly describe the effects of lead on recruitment and differentiation of osteoblasts. It is clear from other studies that lead may alter the differentiation of glial cells and neuroblastoma cells in culture and neurons *in vivo* (*51–53*). The effects of lead on protein, RNA, and DNA synthesis are conflicting. Lead is reported to have little effect, inhibit, or even stimulate various aspects of nucleotide metabolism (*54*). These conflicting results may be the result of methodological differences or phenotypic differences among studies. It is clear, however, from studies using purified DNA and RNA polymerases that lead has the potential to inhibit these pathways. Considering the importance of cell division and differentiation in maintaining osteoblastic function, these processes should be investigated in greater detail in the skeletal system.

Synthesis and Secretion of Bone Matrix

The organic bone matrix, or osteoid, is composed of a large number of constituents, including many proteins synthesized by bone cells, some of which are discussed below. Osteoid also contains many constituents derived from blood such as α_2 heparan sulfate glycoprotein, albumin, several immunoglobulins, and transferrin. Nonprotein constituents include histones, peptides, and an array of lipids. In addition, nearly 20% of the noncollagenous matrix remains unidentified, and the interested reader is directed to recent reviews (*28,55*). Very few of these matrix components have been specifically investigated in lead intoxication.

Collagen

Type I collagen is by far the most abundant organic component of osteoid. Collagen is synthesized by osteoblasts and is assembled outside the cell in a manner similar to other cell types. The effect of lead on type I collagen has not been specifically investigated in a systematic manner. However, several investigations have reported that lead exposure impairs collagen synthesis by bone and other cells. The most direct evidence is the early work by Hass and co-workers who reported an inhibitor effect of lead on vitamin D-stimulated matrix production in bone and arteries in the rabbit (*20*). The inhibition of collagen synthesis by lead has been confirmed by subsequent investigations using human synovial cells, cultured embryonic chick bone, skin, or cultured mouse fibroblasts (*56–59*). Again, the dose-response relationships and clinical significance remain to be established.

Osteocalcin

Osteocalcin (bone Gla protein) is a major noncollage-

nous constituent of bone accounting for 1 to 2% of total bone protein. This acidic, calcium binding protein contains three residues of γ-carboxyglutamic acid per molecule, which is a posttranslational modification dependent upon vitamin K. Osteocalcin is synthesized only by osteoblasts and is mainly secreted into osteoid where the osteocalcin binds to hydroxyapatite. This binding is increased by the binding of Ca^{2+} to osteocalcin (*60*). Although molecular mechanisms are not clear, osteocalcin plays a major, possibly nucleation, role in the normal mineralization process in bone (*61*). Small amounts of osteocalcin found in serum and circulating levels of osteocalcin reflect physiological and pathological states of high bone turnover such as pregnancy, lactation, osteoporosis, and Paget's disease, in which bone turnover rates are altered (*62*). The synthesis of osteocalcin is stimulated by 1,25-dihydroxyvitamin D_3 *in vivo* and *in vitro*.

Plasma levels of osteocalcin are depressed in lead toxic children who had positive EDTA provocative tests. Within a few weeks following in-hospital EDTA chelation therapy, the plasma levels of osteocalcin return to normal (*15*). The mechanism of this reduction of circulating osteocalcin is not clear; the reduction may be *a*) the end result of decreased circulating 1,25-dihydroxyvitamin D_3, *b*) increased osteocalcin degradation, or *c*) reduced synthesis by osteoblasts. Recent studies to elucidate the mechanism of reduced osteocalcin using the ROS 17/2.8 osteoblastlike cells have established that lead is a potent inhibitor of 1,25-dihydroxyvitamin D_3-stimulated osteocalcin synthesis (*63*). In addition, preliminary studies indicate that Pb^{2+} readily displaces Ca^{2+} from osteocalcin and that the binding of lead to osteocalcin impairs binding of this protein to hydroxyapatite (*15*). The functional significance of these findings in children remain to be established, but it is reasonable to suggest that reduced osteocalcin levels reflect reduced osteoblastic activity in lead intoxicated children, and that this lesion may be reversed following successful chelation therapy.

Osteopontin

At least two important bone sialoproteins have been characterized in bone matrix. The bone sialoprotein I (osteopontin) is found only in bone and binds strongly to hydroxyapatite and contains amino acid sequences identical to the cell-binding sequence in fibronectin. No data are available regarding the effects of lead on the synthesis or function of this protein in bone. However, it is interesting to note that in rat brain, lead delays the progression of the highly sialated embryonic form of the neural cell adhesion molecule (N-CAM) to the less sialated adult form (*64*).

Osteonectin

Osteonectin is a bone protein with the unique ability to facilitate *in vitro* mineralization of type I collagen due to a high binding affinity for both apatite and insoluble collagen. It appears that this protein plays a role in initiating mineral deposition and in linking apatite to the collagenous bone matrix. Currently, there is no information concerning the effects of lead on the production or function of osteonectin.

Little information is available, either directly or by extrapolation from other tissues, regarding the effects of lead on the diverse organic components of the bone matrix. This lack of information is due, in part, to the fact that biochemical functions of these constituents are only now being clarified. Further development and application of cDNA probes, fluorescent antibodies, and other molecular probes of bone cell function should help us establish the functional role of individual components of the bone matrix. Once the normal regulation and function of the constituents of the osteoid are defined, the interactions and effects of lead on the organic bone matrix can be understood.

Direct Effects of Lead on Osteoclasts

Osteoclasts resorb bone during development, maintenance, and repair of the skeleton. In addition, osteoclasts elaborate paracrine factors that influence osteoblastic proliferation, migration, differentiation, matrix synthesis, and cessation of synthesis (*65–68*). These large, multinuclear cells are located on bone surfaces next to large vascular channels. Although osteoclasts may be longer lived than osteoblasts, it appears that the lifetime of an individual nuclei is 10 to 20 days and that the osteoclasts are renewed by fusion of preosteoclasts (*69*). Although the exact mechanisms of bone resorption are not clear, several processes involved in resorption may be important targets of lead toxicity or modifiers of bone lead metabolism. Bone resorption involves both exocytosis and endocytosis at the ruffled border. Acid hydrolases are synthesized and secreted into the space between the osteoclast and the mineralized bone matrix. This space is acidified to provide an optimal, confined environment for the function of hydrolases, thus facilitating demineralization. Cell respiration provides the energy and substrates for carbonic anhydrase isozyme II and the Na^+,K^+-ATPase, which supply and pump H^+ to the confined extracellular compartment below the ruffled border. The organic and inorganic products of resorption are taken into the osteoclast by endocytosis, further digested in secondary lysosomes, and finally released into venous sinuses.

Lead Inclusion Bodies

Lead inclusion bodies commonly occur in the cytoplasm and nuclei of osteoclasts, but not osteoblasts or osteocytes (*70,71*). The ultrastructural characteristics of these inclusions are indistinguishable from lead inclusions formed in the renal tubular cells, hepatocytes, and astrocytes. In fact, Hamir et al. (*72*) found that acid-fast inclusions were more common in osteoclasts

(95% of cases) than in liver or kidney (37 and 68%) of lead poisoned dogs. The presence of these inclusions in any cell type is considered pathognomonic for lead intoxication. Microscopic studies suggest that osteoclasts are more sensitive to overt toxic effects than osteoblasts or lining cells (*73,74*). However, it is not established if the toxic effects are due to the direct actions of lead on osteoclasts or mediated indirectly though lead effects on osteoblasts, thereby impairing osteoclast function.

The principal protein component of the inclusion body in the kidney is the low molecular weight α_2-microglobulin (*75*). This relatively high-affinity, lead-binding protein mediates the translocation of lead into isolated nuclei (*76*). In addition to mediating the intranuclear bioavailability and effects of lead, this protein may modulate the toxicity and the bioavailability of lead to target enzymes, including as δ-aminolevulinic acid dehydratase (*77,78*) and the distribution of lead in kidney cells (*79*). The molecular mechanisms involved in induction of α_2-microglobulin in renal tubular cells and the normal biochemical function and toxicological significance of the protein are the subject of active investigation (*75*). No comparable biochemical characterization of lead inclusion bodies and lead binding proteins has been undertaken in bone cells, nor is it clear why the osteoclast is the only bone cell to form these lead inclusions. The osteoclast may be exposed to or take up more lead during the process of bone resorption.

Bone Resorption

Impaired bone resorption, which is consistent with inhibition of osteoclastic function, is a consistent generalization that can be inferred from the relatively few studies that have examined bone structure as affected by lead toxicity. The mechanism of inhibited osteoclastic function may be mediated through *a*) the effects of lead on 1,25-dihydroxyvitamin D_3 and other systemic regulators of bone resorption, *b*) overt toxicity and cellular death of osteoclasts (*21,71*), *c*) the result of more local and specific effects of lead on osteoclast function, including uncoupling of osteoblastic regulation, and *d*) a combination of the above actions. It is likely that all of these processes contribute to impaired osteoclastic bone resorption in varying degrees, depending upon duration and severity of lead exposure.

Carbonic Anhydrase II and Na^+,K^+-ATPase

Carbonic anhydrase II, which provides the protons to acidify the confined extracellular space, is located just inside the ruffled border of the osteoclast. This enzyme contains Zn^{2+} at the active site, and like some other zinc metalloenzymes such as δ-aminolevulinic acid dehydratase, carbonic anhydrase II is sensitive to inhibition by lead. Although no investigations have been performed to date using osteoclasts, several studies with purified apoenzyme have demonstrated binding of lead to the active site of the enzyme, resulting in reduced hydrolysis of carbon dioxide and thus reduced production of protons (*80,81*).

Na^+,K^+-ATPase, located on the ruffled border membrane, provides the proton motive force to move protons from the cytoplasm to the extracellular space apposed to the mineral matrix. Although it has not been examined specifically in osteoclasts, analogies to other tissues suggest two mechanisms by which lead might interfere with the movement of protons across the plasma membrane. First, lead may directly inhibit Na^+, K^+-ATPase, as has been reported in kidney and brain (*82–84*). Second, through its effects on cellular energy metabolism, lead may also impair acidification by reducing availability of ATP substrate (*85–87*).

Lead would be available to interact with carbonic anhydrase II and Na^+,K^+-ATPase from at least three sources, including blood, osteoclast lead stores (*88*), and lead released following resorption of mineralized matrix. The result of decreased acidity in the extracellular space would be a suboptimal environment for activity of the acid hydrolases and therefore impaired bone resorption at that site consistent with the pathological changes described above.

Effects of Lead on Osteocytes and Lining Cells

As new bone is formed, some osteoblasts are buried in bone and become osteocytes. These cells reside in lacunae and are connected to each other by thin cell projections in canaliculi. Osteocytes can both make and resorb bone lining the lacunae, but probably function mainly in calcium homeostasis rather than bone repair and maintenance. Lining cells are thin, flat, elongated cells which cover most bone surfaces that are not active, that is, bone undergoing neither formation nor resorption. The function of these cells is not agreed upon, but it is believed that the lining cells may function as progenitors for osteoblasts, serve as a selective barrier between the bone matrix and the vasculature, or regulate hydroxyapatite crystal growth (*28*).

Little is known regarding the effects of lead on osteocytes and lining cells for two reasons. First, the lack of a clearly established function performed by these cells makes it very difficult to establish lead-dependent malfunction. Second, in regard to osteocytes, it is very difficult to remove the cells from the mineralized bone matrix for study *in vitro*. It should be noted that electron microscopic studies suggest that osteocytes are less likely to show ultrastructural evidence of cell injury than osteoclasts and osteoblasts (*70,71*).

Cellular Metabolism of Lead

In actuality, the metabolism of lead in bone cells cannot be viewed independently of lead metabolism in the mineral bone matrix. In fact, the experimental and conceptual problem is to characterize one in the pres-

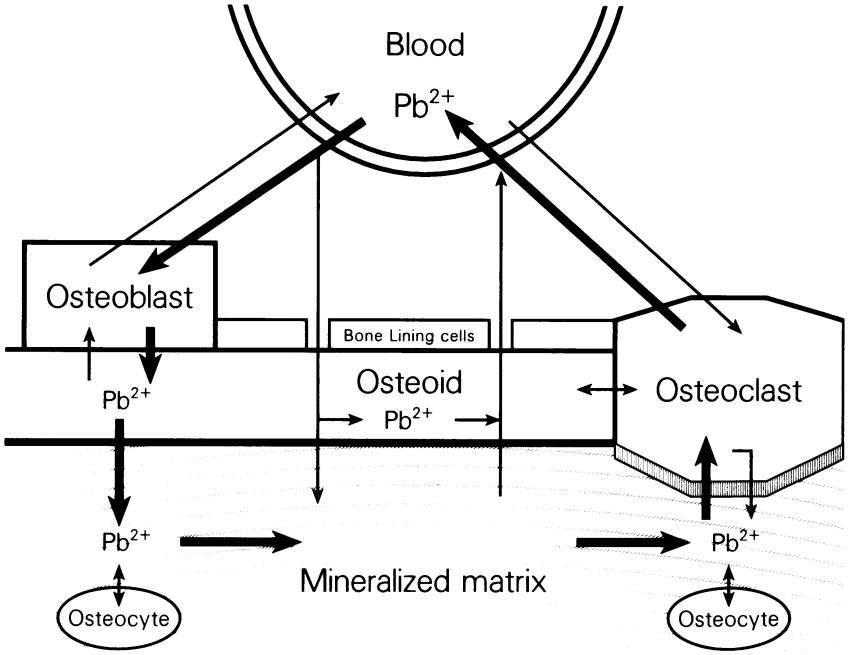

FIGURE 3. Schematic figure illustrating the biological and chemical pathways by which lead is deposited and removed from bone. The systemic and local regulators of bone cell function are illustrated in Figures 1 and 2.

ence of the other and to determine the relative contribution of biological and chemical processes to bone lead metabolism. Figure 3 illustrates the relationship of some of the more important pathways for lead in bone. Movement of lead into bone is predominantly mediated by osteoblast activity, while osteoclast activity is mainly responsible for loss of lead from bone. Some aspects of this scheme have been characterized in detail as discussed below, while others are speculative. More complete descriptions of skeletal lead metabolism are given elsewhere (*3,89–94*).

It is important to note at the onset that lead readily displaces Ca^{2+} by cation exchange processes in the hydroxyapatite crystal in both natural and synthetic apatite. It may also be useful to note that the position of lead in the hydroxyapatite crystal has been studied by several methods including X-ray diffraction and that Pb^{2+} occupies both Ca^{2+} sites in the crystal lattice (*95–97*). The effect of Pb^{2+} on the crystallinity or resorption of bone remains to be determined. In addition, lead is a potent calcergen, or inducer of ectopic calcification. Subcutaneous injection of lead produces an accumulation of lead, probably in the form of lead triphosphate, around the connective tissue, which eventually gives way to apatite formation and can be prevented by high levels of calcitonin (*98,99*). In addition, calcification of soft tissue is sometimes associated with lead intoxication (*100*). The significance and prevalence of these effects is not established.

The cellular and subcellular metabolism of lead has been characterized in diverse cell types using several approaches including radiotracer techniques, autoradiography, histochemistry, electron microprobe energy dispersive X-ray spectroscopy, and differential centrifugation. To some extent, the subcellular distribution of lead depends on the method of study (*101*). Relatively few of these approaches have been applied to the investigation of lead in bone cells.

The first characterizations of bone lead metabolism *in vitro* were conducted using bone organ cultures of fetal rat radius and ulna (*102–104*). These studies showed that at least one compartment of total bone lead was readily exchanged and was modulated by the same ions and hormones that regulate bone calcium metabolism. Subsequent studies in primary cultures found that the osteoclast accumulated considerably more lead than osteoblasts from the culture medium (*88, 105*). As indicated above, lead is accumulated in inclusion bodies by osteoclasts, but not osteoblasts or osteocytes. The most complete description of cellular lead metabolism is the compartmental modeling of ^{210}Pb washout curves in primary cultures of osteoclastic (*88*) and osteoblastic (*105*) bone cells isolated from fetal mouse calvaria (*88,105,106*). Each cell type contains three distinct kinetic pools of intracellular lead (Figs. 4 and 5). The majority of the lead is associated with a slowly exchanging pool which probably includes the nitochondrial lead. The remaining lead is distributed in two pools, one of which exchanges across the plasma membrane with a half-time of approximately 1 min. The biological constituents of these two more rapidly exchanging pools remain enigmatic and undoubtedly include lead associated with a wide variety of biochemical and structural constituents of cells.

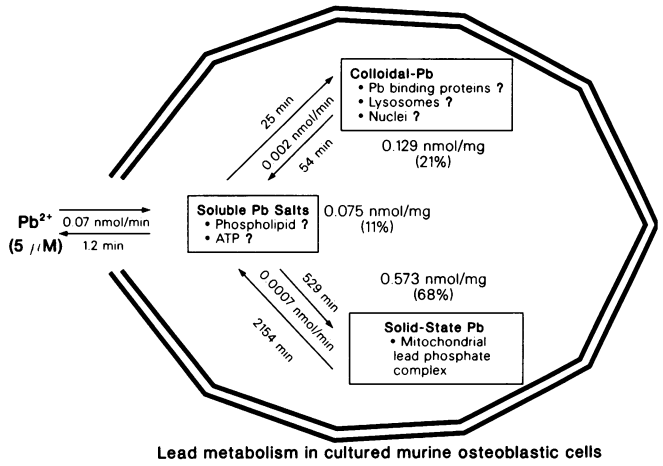

FIGURE 4. Physiological model for the steady-state metabolism of lead in cultured murine osteoblastic bone cells. All data are normalized to 1 mg of cell protein, with pool sizes expressed as nanomoles lead per milligram of protein and percent of total cell lead, fluxes as nanomoles per milligram cell protein per minute, and half-times as minutes. From Long et al. (*105*).

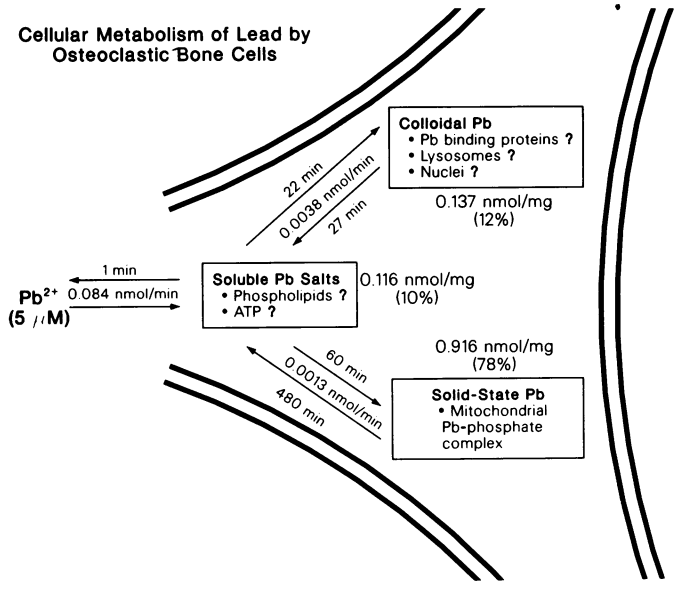

FIGURE 5. Physiological model for the steady-state metabolism of lead in cultured murine osteoclastic bone cells. All data are normalized to 1 mg of cell protein, with pool sizes expressed as nanomoles lead per milligram of protein and percent of total cell calcium, fluxes as nanomoles per milligram cell protein per minute, and half-times as minutes. From Pounds and Rosen (*88*).

These same studies also found that hormonal regulation of these cells caused a redistribution of Pb^{2+} (and Ca^{2+}) inside the cell, usually without altering total cellular lead. This dynamic redistribution at the subcellular level may be related to impaired hormone responsiveness of these cells.

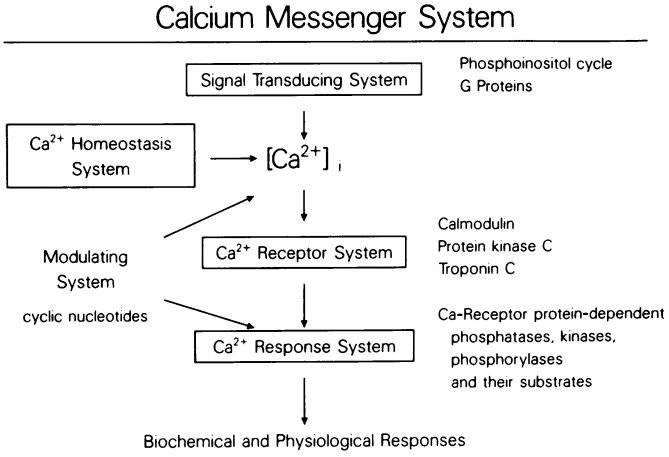

FIGURE 6. A schematic representation of the calcium messenger system illustrating the relationships of the signal transducing, Ca^{2+} homeostasis, Ca^{2+} receptor, Ca^{2+} response, and modulating systems. From Pounds (*114*).

Lead-Calcium Interactions in Bone Cells

Lead-calcium interactions occur at all levels of biological organization, i.e., systemic, cellular, subcellular, and molecular. The Ca^{2+} messenger system may be considered as the integrated function of several component sub-systems (Fig. 6). Each of these component parts will be briefly described including a brief illustration or summary of the action of lead on each subsystem. A full review of these interactions is beyond the scope of this forum, and the reader is referred to more complete descriptions of the calcium messenger system (*107–109*) and of the significance of these lead-calcium interactions in lead toxicity (*22,110–113*).

The calcium-dependent cell functions that might be of most importance to skeletal toxicity of lead include exocytosis of systemic regulators by endocrine cells, cell division, ameboid movement and differentiation of osteoprogenitor cells, signal-response coupling of hormonal and local signals in osteoblasts and osteoclasts, and substitution of lead for calcium in the calcium binding proteins of the osteoid. The hormonal regulation of bone cell function was believed until recently to be dominated by the cAMP messenger system. However, application of newer $[Ca^{2+}]_i$ probes and availability of well-characterized, bone-derived cell lines has demonstrated that the biochemical responses of osteoblasts (and thus local regulation of osteoclast response) to stimulation by parathyroid hormone and 1,25-dihydroxyvitamin D_3 are regulated by calcium and cAMP in a complex manner (*115–119*). Although many aspects of the role of the calcium messenger system in mediating systemic and local regulation are under active investigation, there are sufficient investigations of lead-calcium interactions in bone cells to warrant conclusions and comparisons to other target cells.

In addition, investigations in other systems might provide insight into observations made in bone cells.

Signal Transducing Systems

The concentration of free cytoplasmic calcium ion, $[Ca^{2+}]_i$, is normally maintained between 50 and 150 nM by the Ca^{2+} homeostasis system. The signal transducing system, which is composed mainly of products from phosphoinositol metabolism, are the second messengers that transduce the hormonal or electrical signal at the plasma membrane to a Ca^{2+} signal by increasing $[Ca^{2+}]_i$ in one or more parts of the cell. Important constituents of the signal transduction system include Ca^{2+} gates in the plasma membrane and the products of phosphoinositol metabolism. Phosphoinositol metabolites, mainly inositol (1,4,5)trisphosphate, transduce and carry the message to mobilize Ca^{2+} from the plasma membrane receptor to the endoplasmic reticulum (*107,108,120*).

Lead interferes with the generation of a Ca^{2+} signal in may cells. Lead blocks Ca^{2+} entry into nerve terminals, thereby inhibiting the Ca^{2+} signal (*121*). This early work was extended by Simons, who demonstrated in adrenal medullary cells that Pb^{2+} inhibited Ca^{2+} entry when calcium channels were opened by depolarization (*122,123*). In fact, the calcium channel had much higher affinity and permeability to Pb^{2+} than to Ca^{2+}. In contrast, cells in which the Ca^{2+} signal is predominantly achieved by redistribution of intracellular calcium do not show the same lead-dependent inhibition of the Ca^{2+} signal (*124*). The effect of lead on the Ca^{2+} signal is complicated by the observation that α-adrenergic stimulation or depolarization, both of which induce a Ca^{2+} signal, also mobilize Pb^{2+}. Thus, a Pb^{2+} signal may be produced concurrently with a normal or a lead-altered Ca^{2+} signal (*122,125*). In addition, hormonal stimulation of osteoclastic cells with calcitonin and simulation of osteoblastic cells with parathyroid hormone or 1,25-dihydroxyvitamin D_3 affected a redistribution of subcellular lead within the three intracellular kinetic pools.

Ca^{2+} Homeostasis System

The Ca^{2+} homeostatic system is a medley of Ca^{2+} pumps and channels that work to keep the free cytosolic calcium ion, $[Ca^{2+}]_i$, within the normal physiological range (*126,127*). Mitochondria serve as a high-capacity, low-affinity Ca^{2+} storage site and contain a Ca^{2+} transporter on the inner membrane to move Ca^{2+} from the cytoplasm to the mitochondrial matrix. This uptake of Ca^{2+} is inhibited by lead in isolated mitochondria or tissue slices. Lead is also taken up and stored in mitochondria via this same transporter. The endoplasmic reticulum is more important in maintaining Ca^{2+} at lower levels than the mitochondria; however, little is known regarding the effect of lead on Ca^{2+} uptake or Pb^{2+} accumulation by the endoplasmic reticulum.

Even in unstimulated cells, steady-state calcium homeostasis is affected in primary cultures of osteoclastic bone cells, osteoblastic bone cells, and a clonal osteoblastic cell line, ROS 17/2.8 cells (*128,129*). Total cell calcium per milligram cell protein is increased with lead intoxication. Most of this additional cell calcium is associated with the mitochondrial calcium pool. These experiments suggest that the plasma membrane of lead intoxicated cells is more permeable to Ca^{2+} and/or less able to extrude Ca^{2+} from inside the cell to the extracellular environment. As the plasma membrane becomes less proficient at maintaining barrier and transport functions, the Ca^{2+} is sequestered in mitochondria in an attempt to maintain Ca^{2+} homeostasis. This conclusion is supported by recent direct measurements of $[Ca^{2+}]_i$ using ^{19}F NMR, also in ROS 17/2.8 cells (*130*). These investigators found that treatment with 5 or 25 μM Pb^{2+} produced concentration-dependent increases in $[Ca^{2+}]_i$ which persisted over 5 hr. These findings in bone cells are consistent with similar studies in cultured rat hepatocytes, bovine brain capillary endothelial cells, and lead intoxication *in vivo* (*22,131*).

Ca^{2+} Receptor System

The Ca^{2+} receptor system is a family of homologous calcium-binding proteins that transduce the intracellular Ca^{2+} signal to biochemical or mechanical responses. The Ca^{2+} receptor proteins include calmodulin, protein kinase C, calcimedins, parvalbumins, troponin-C, and many others (*107,108,132–134*). Some of these Ca^{2+} receptor proteins, such as troponin C, are specific to certain cell types, while others are ubiquitous. The two most versatile and ubiquitous Ca^{2+} receptor proteins are calmodulin and protein kinase C. The calmodulin-Ca^{2+} complex activates calmodulin-dependent protein kinases and a variety of other enzymes to elicit responses. The calmodulin-mediated responses are typically of either brief duration (seconds) or represent the initial phase of a more sustained response. Well-characterized calmodulin-dependent responses include neurotransmitter release, endocrine and exocrine secretion, muscle contraction, etc. Protein kinase C is activated by Ca^{2+} and a lipid metabolite produced by the Ca^{2+} signal transduction system, diacylglycerol. Protein kinase C activates protein kinases and phosphatases with both broad and narrow spectrum of protein substrates (*134*). Protein kinase C-mediated responses are typically of longer duration than calmodulin-mediated responses and include initiating cell division and proliferation, cell-cell communications, organization of the cytoskeleton, and many others (*106,107*).

Lead can perturb the Ca^{2+} receptor system directly by substituting for Ca^{2+} with either more or less activity, or indirectly by interfering with the generation or removal of the Ca^{2+} signal. Pb^{2+} will effectively and functionally displace or substitute for Ca^{2+} in each of these receptor proteins (*22,135–139*). The weight of evi-

dence at this time, from these and other studies, indicates that a Pb^{2+}-calmodulin or Pb^{2+}-protein kinase C complex activates the same spectrum of enzymes and elicits a similar biochemical response as the Ca^{2+} protein complex. However, these proteins may have a higher affinity for Pb^{2+} than Ca^{2+}, so the prolonged Ca^{2+}-mediated response seen may be due to a Pb^{2+} signal.

The increase in total cell calcium increases in transplasma membrane fluxes and mitochondrial calcium in osteoclasts and osteoblasts (*128,129*) along with increases in $[Ca^{2+}]_i$ in osteoblastic cells may be explained by Pb^{2+} activation of protein kinase C. Picomolar concentrations of Pb^{2+} activate protein kinase C in the absence of Ca^{2+} (*137*). Osteoblastlike cells contain a protein kinase C-activated calcium channel which could be opened by interaction with Pb^{2+}. As a result of opening, or partial opening of the calcium channels, Ca^{2+} would flow down the electrochemical gradient from outside the cell into the cytoplasm. $[Ca^{2+}]_i$ would be increased, and the mitochondria would accumulate Ca^{2+} in an attempt to restore low $[Ca^{2+}]_i$. Steady-state flux across the plasma membrane would be increased, influx due to increased membrane permeability to Ca^{2+}, and efflux due to increased Ca^{2+}-ATPase activity.

Ca^{2+} Response Systems

The Ca^{2+} signal is transduced by Ca^{2+}-receptor proteins to a biochemical or mechanical response. Mechanical responses include contraction and cell movement, while the biochemical responses encompass the range of cell functions of cells including cell differentiation and proliferation. Biochemical responses are often elicited by the action of calmodulin- or protein kinase C-dependent kinases and phosphorylases. These enzymes include cyclic nucleotide phosphodiesterase, phosphorylase kinase, adenylate cyclase, multiprotein kinase, calmodulin-dependent kinase, Ca^{2+}-ATPase, and many others (*134,140,141*). Because these processes are somewhat distal from the $[Ca^{2+}]_i$ signal, it is often difficult to experimentally establish the lead-dependent perturbation of the Ca^{2+} response system with suitable scientific rigor.

Modulating Systems

The calcium messenger system cannot be considered either conceptually or experimentally as an isolated target pathway because other messenger systems, mainly cyclic nucleotides, modulate or counter-regulate the calcium signal and calcium-mediated responses. At the simplest level, Ca^{2+} and cAMP signals counter-regulate each other: The Ca^{2+}-calmodulin activates phosphodiesterase, the enzyme responsible for degradation of cyclic nucleotides, whereas cAMP and cGMP often active Ca^{2+}-ATPases, thereby reducing the $[Ca^{2+}]_i$ signal. In fact, the interaction of the two messenger systems is somewhat more complex in that parathyroid hormone elicits both a Ca^{2+} and cAMP response in osteoblasts, which illustrates that some pathways may be activated by both systems (*117,118*). The generation and regulation of the cAMP signal is much simpler than the generation and regulation of the $[Ca^{2+}]_i$ signal because the enzyme adenylate cyclase makes cAMP and the enzyme phosphodiesterase degrades cAMP.

The effects of lead on the cAMP messenger system have not been systematically characterized. Limited studies have shown that lead inhibits adenylate cyclase and thereby the generation of cyclic nucleotides (*142*), although increased baseline levels of cAMP have been reported in tissues of lead intoxicated rats (*143*). The impaired generation of a cAMP signal could be exacerbated by Pb^{2+}-calmodulin dependent activation of phosphodiesterase, thereby degrading cyclic nucleotides (*136*). In actuality, the spatial and temporal aspects of cAMP and Ca^{2+} signals are so complex as to preclude prediction of toxicity based on simple observations in cell-free systems (*113,114*). In fact, studies of parallel cAMP- and Ca^{2+}-mediated hormonal inhibition of pyruvate kinase found no effects of lead on Ca^{2+}-mediated processes, but not cAMP processes (*144*).

Thus, lead has diverse and complex actions on the calcium messenger system, emphasizing the importance of this pathway as a key molecular and cellular target of lead toxicity. Further work must be done to clarify the contributions of direct and indirect effects of lead on the calcium messenger system in mediated toxicity (Table 1). Although many effects of lead on Ca^{2+} homeostasis are described, the clinical significance and relationship of these changes in Ca^{2+} metabolism to the experimental and clinical manifestations of skeletal lead toxicity in bone and other tissues remains to be clearly defined.

Table 1. Spectrum of known actions of lead on Ca^{2+} homeostasis and Ca^{2+}-mediated processes at the cellular and molecular level.[a]

Interaction	Ca^{2+} signal transduction and homeostasis	Ca^{2+}-receptor and response systems
Direct	Plasma membrane Ca^{2+} channels Mitochondrial Ca^{2+} pump Ca^{2+}-ATPase	Calmodulin Protein kinase C Troponin C Osteocalcin Calbindin Oncomodulin
Indirect	Adenylate cyclase Na^+,K^+-ATPase	Adenylate cyclase
Secondary	Hydrolysis of ATP Decreased heme Protein-sulfhydryl binding	Protein-sulfhydryl binding

[a] Most of these cellular and molecular actions of lead have been well established in several *in vitro* and *in vivo* studies using diverse biological systems. The difficulty is separating the direct from the less direct actions and experimentally characterizing those actions as key and obligatory events in chronic lead toxicity. Modified slightly from Pounds (*114*).

Summary

The toxicity of lead in the skeleton is like that produced in other target tissues in that lead does not cause a unique disease or pathological lesion, but rather decremental loss of organ function manifested by many biochemical, molecular, and structural lesions. The effects of lead on the skeleton may be produced through two general processes. First, effects may be indirect and secondary to lead effects on the endocrine organs which synthesize or produce hormones regulating bone function and bone mineral metabolism. Second, lead may directly perturb bone cell function by *a*) producing overt toxicity and cell death in bone cells, *b*) interfering with essential cell process including cell division, motility, and enzyme function, and *c*) by altering stimulus-response coupling, and/or osteoblast-osteoclast coupling through effects on the calcium messenger system. In fact, lead toxicity in bone is likely to be the sum of these effects. Regardless of the site of action of lead, whether direct, indirect, or both, the effect of lead in bone is ultimately expressed by bone cell dysfunction. Thus, understanding the interactions of lead with bone cells is central to toxicological and clinical significance of bone lead metabolism. A clear understanding of the cellular processes should provide important insights for identifying populations at risk to redistribution of lead from the skeleton and the bone functions which are most sensitive to lead toxicity.

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